## Presented at the BIG4 Workshop "3D Imaging and Innovative Approaches in Biosystematics" 8-13 May 2017, Vienna

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MicroCT gives an inside view



Orcula dolium
Terrestrial gastropod
SkyScan 1174,
7.7 µm voxel size.



### Multi-scale imaging, non-destructive

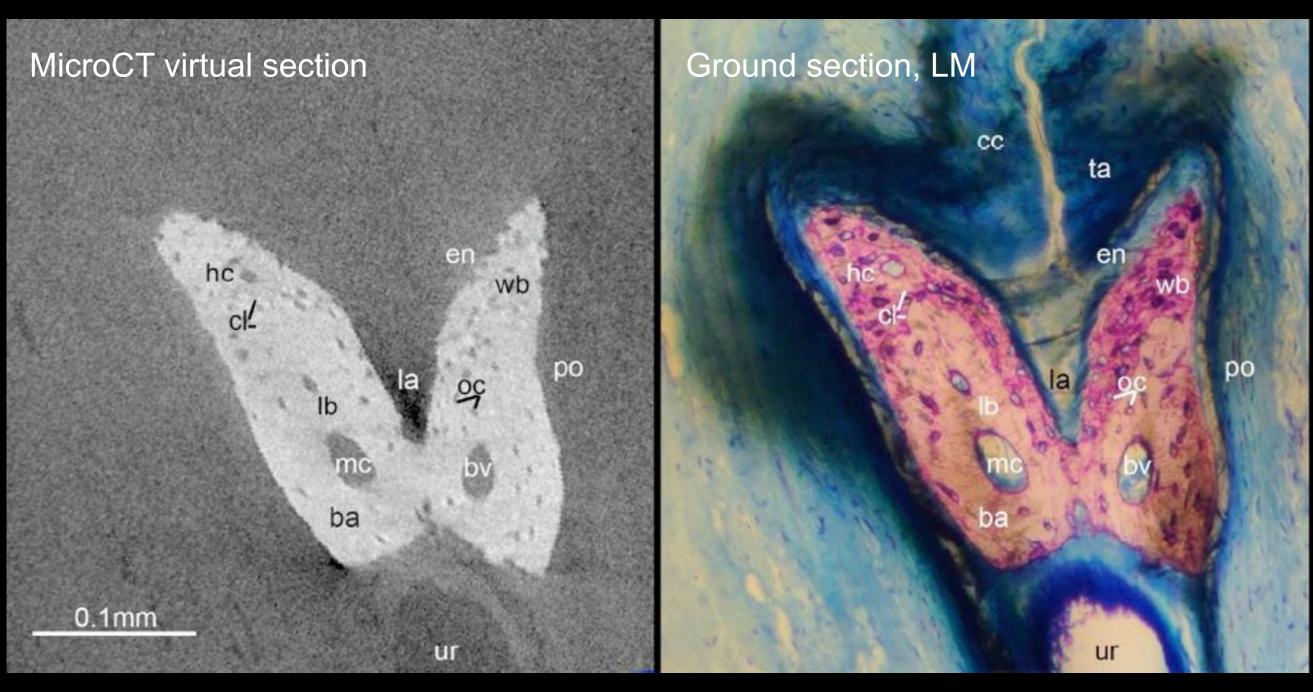


Xradia MicroXCT

Work of Anna Nele Herdina, Theoretical Biology, UniVie

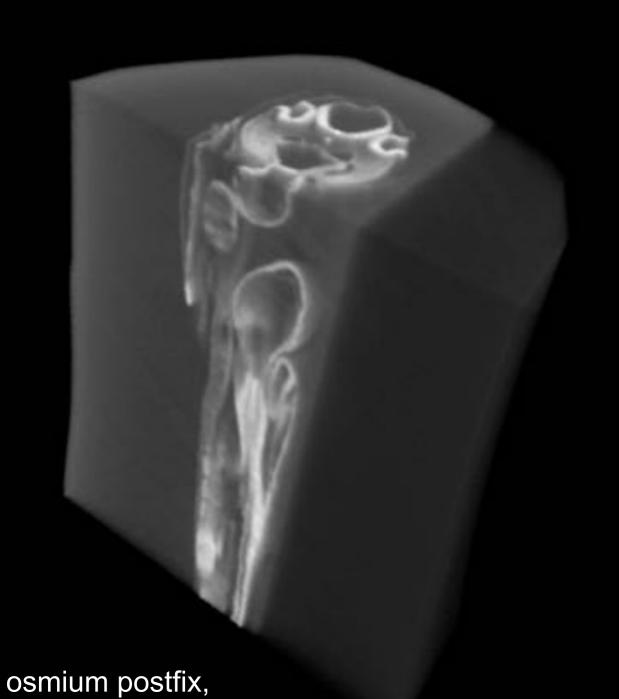
#### Correlative imaging

### Ground sections to calibrate microCT images: Bat baculum histomorphology



Nele Herdina et al. in press, J. Morph.

### Samples prepared for TEM can be scanned in the block



80 kVp





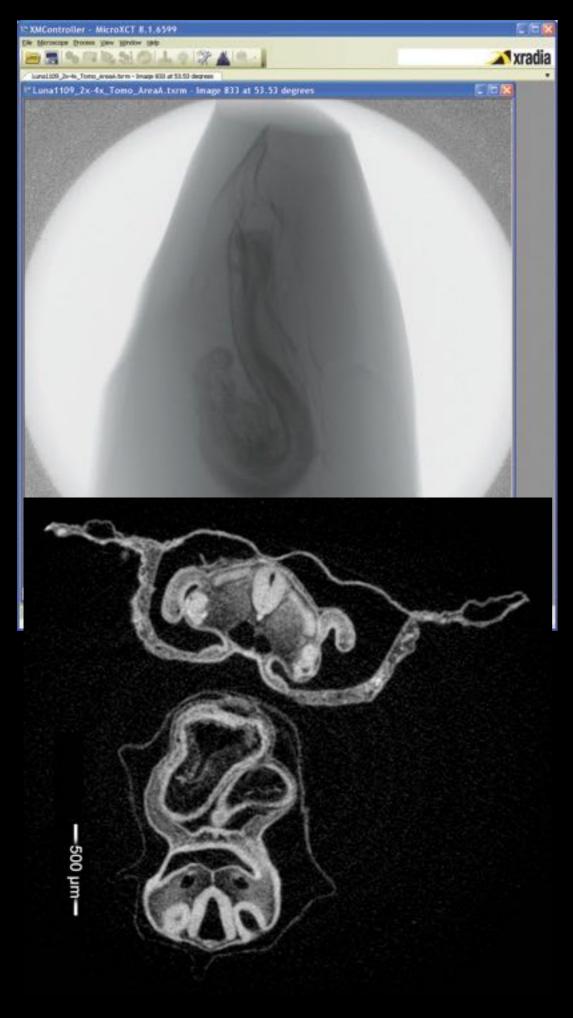
### Cross-platform imaging

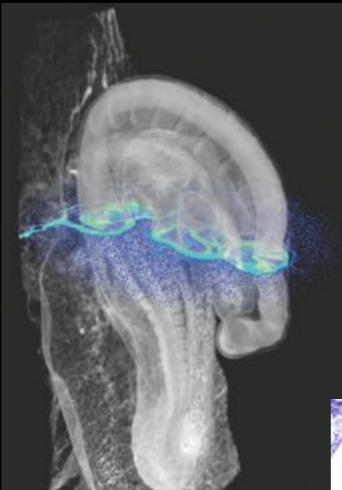
MicroCT

Light microscopy

TEM

Collaboration with Stephan Handschuh and Ingrid Walter, Vet Med Uni Vienna.

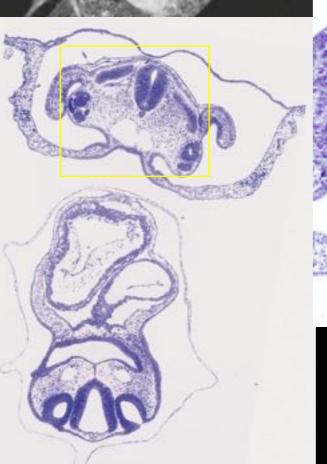




### Cross-platform imaging

Horse embryo, 21 days

With Stephan Handschuh and Ingrid Walter, Vet Med Uni Vienna.



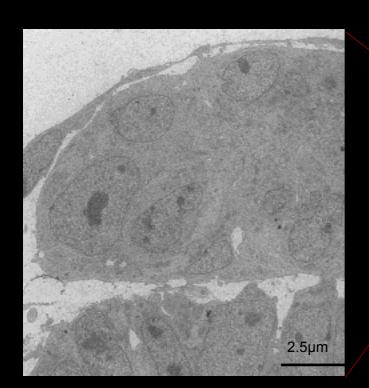
Sections of the same block: semi-thin and toluidine blue for histology

#### METHODOLOGY

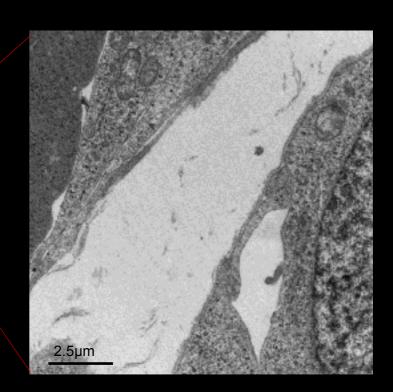
Open Access

A correlative approach for combining microCT, light and transmission electron microscopy in a single 3D scenario

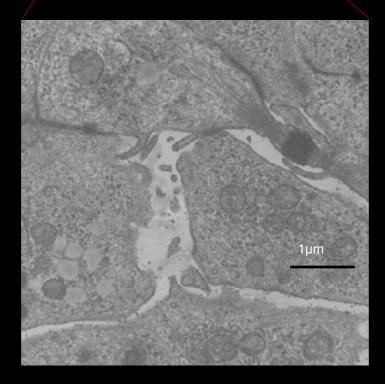
Stephan Handschuh<sup>1,2,3\*</sup>, Natalie Baeumler<sup>4</sup>, Thomas Schwaha<sup>5</sup> and Bernhard Ruthensteiner<sup>4</sup>







TEM of pronephros (kidney) development, with its 3D context



# Other projects: Cell-type-specific imaging



Selective contrasting of melanocytes in zebrafish using autometallography

### Imaging molecular probes



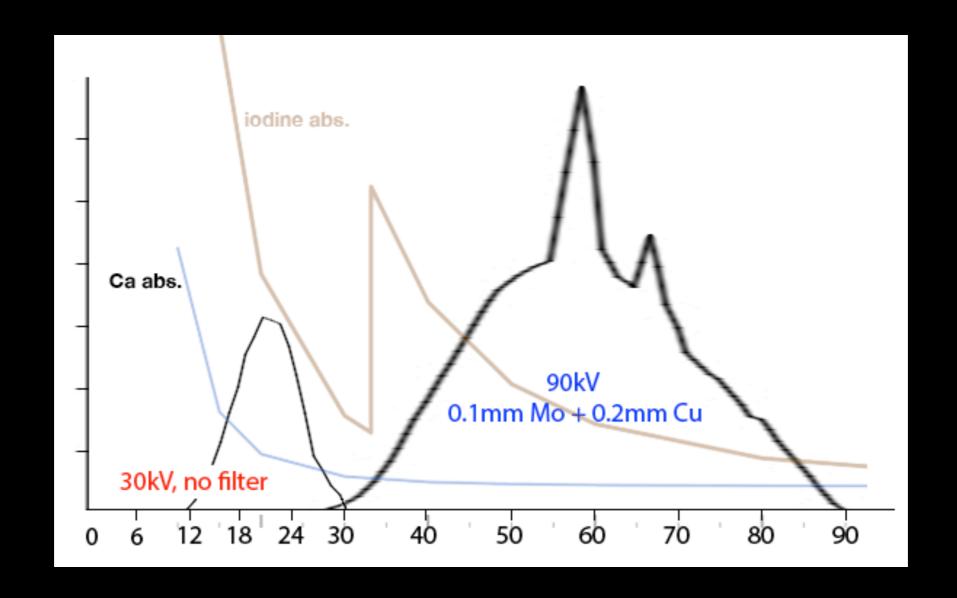
Acetylated alpha-tubulin, st. 23 chick

### Imaging molecular probes with microCT



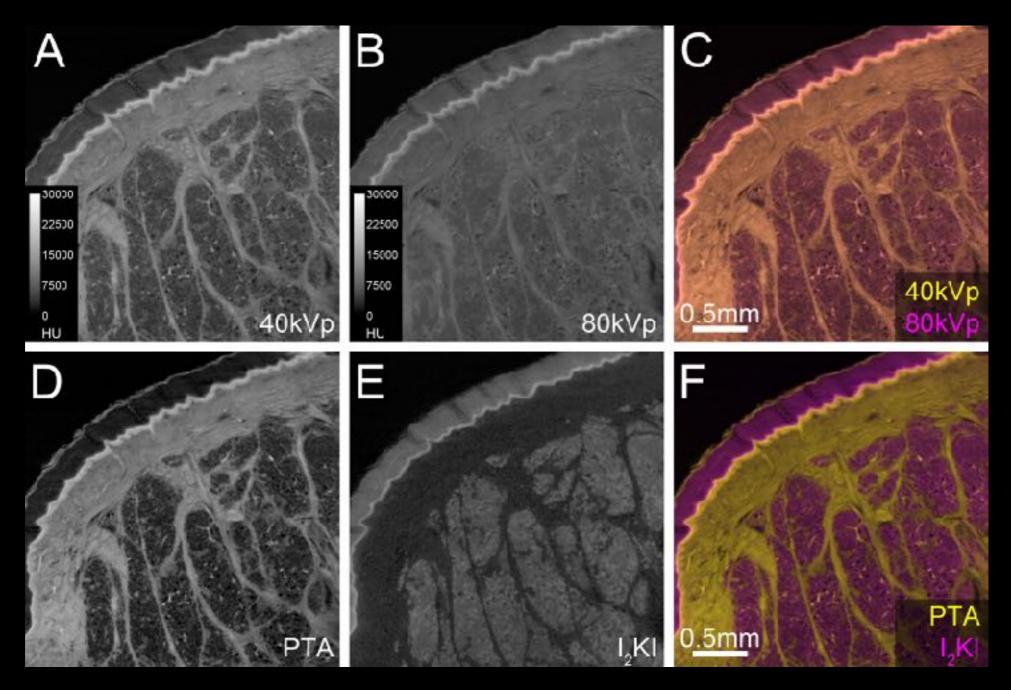
Acetylated alpha-tubulin, st. 23 chick

## Dual-energy microCT can distinguish different materials in the sample



Handschuh S, Beisser CJ, Ruthensteiner B and Metscher BD. 2017. Microscopic dual energy CT (microDECT): a flexible tool for multi-channel ex vivo 3D imaging of biological specimens. Journal of Microscopy, In press.

## Dual-energy microCT can distinguish different materials in the sample



Double-stained cat digit pad, collagenous and adipose tissues.

Handschuh S, Beisser CJ, Ruthensteiner B and Metscher BD. 2017. Microscopic dual energy CT (microDECT): a flexible tool for multi-channel ex vivo 3D imaging of biological specimens. Journal of Microscopy, In press.

### Meshes and models from 3D images

### Snapshots, scale bars in Fiji & Amira

and filters

#### Other free 3D image software

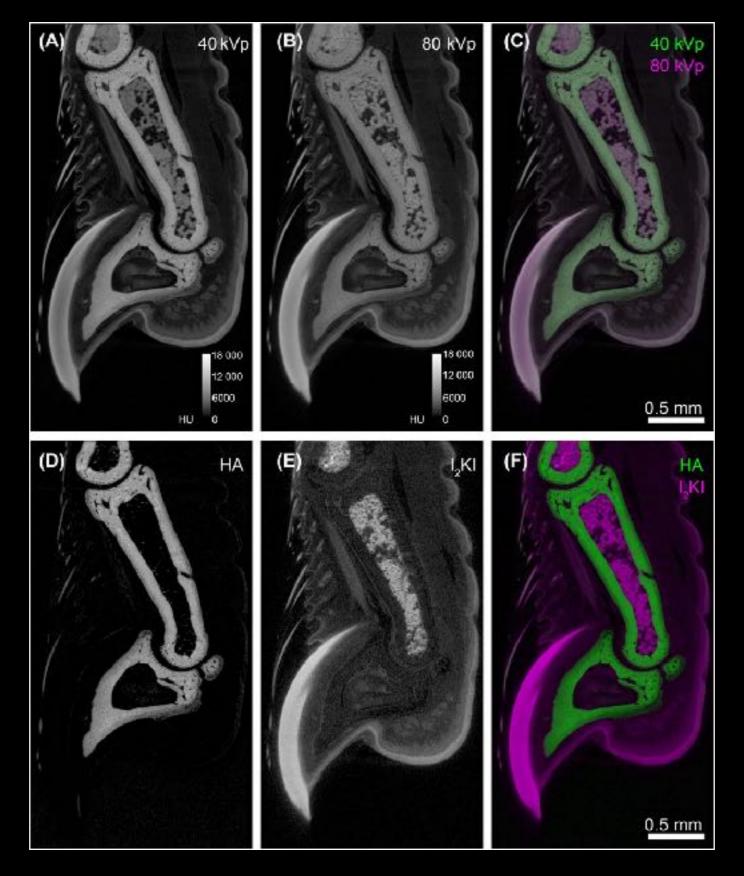
check out <a href="mailto:idoimaging.com">idoimaging.com</a>

#### Images communicate: what does your picture say?



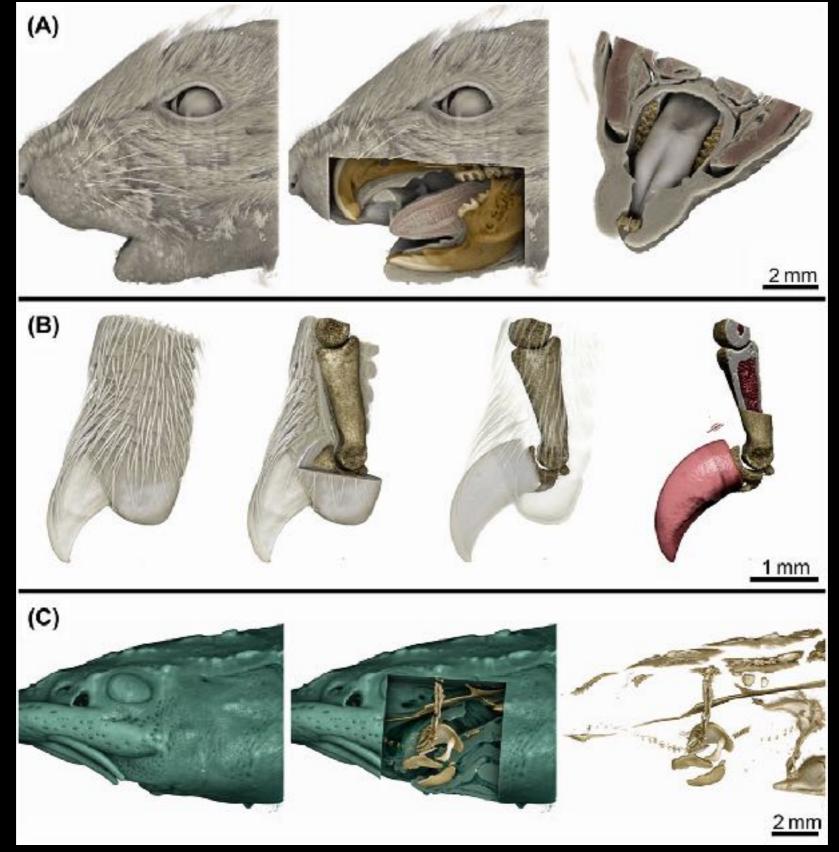
van Soldt et al., 2015, PLoS ONE 10

#### Images communicate: what does your picture say?



Handschuh et al 2017. J Microsc, In press.

#### Images communicate: what does your picture say?



Handschuh et al 2017. J Microsc, In press.

Cybertype: a virtual specimen that helps define a species – like *Ommatoiulus avatar* 



#### Cybertypes provide online access to 3D micromorphology



http://www.datadryad.org/resource/doi:10.5061/dryad.2pf38







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Data from: A new dimension in documenting new species: high-detail imaging for myriapod taxonomy and first 3D cybertype of a new millipede species (Diplopoda, Julida, Julidae)



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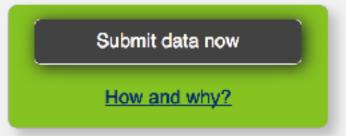
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Title O-avatar\_holotype\_gonopods\_I2E\_1-9um\_PNG

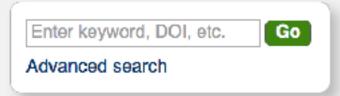
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#### Search for data



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## Cybertype: a virtual specimen that helps define a species – like *Ommatoiulus avatar*



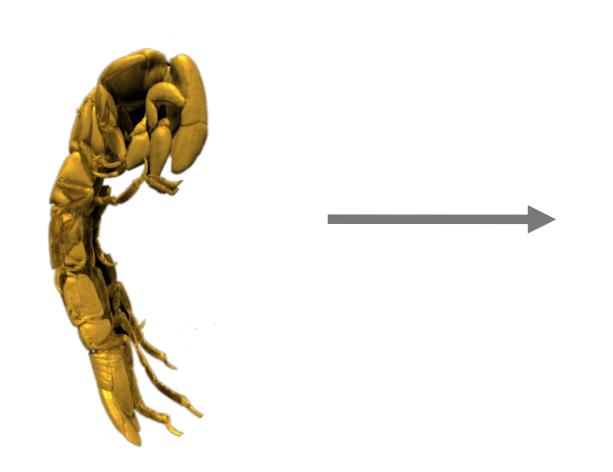
(2015). *PLoS ONE* **10**(8): e0135243.

#### Image ethics: publish responsibly

- D. W. Cromey: Ethical guidelines for the appropriate use and manipulation of scientific digital images
  - 1. Scientific digital images are data that can be compromised by inappropriate manipulations.
  - 2. Manipulation of digital images should only be performed on a copy of the unprocessed image data file (Always keep the original data file safe and unchanged!).
- 3. Simple adjustments to the entire image are usually acceptable.
- 4. Cropping an image is usually acceptable.
- 5. Digital images that will be compared to one another should be acquired under identical conditions, and any post-acquisition image processing should also be identical.
- 6. Manipulations that are specific to one area of an image and are not performed on other areas are questionable.
- 7. Use of software filters to improve image quality is usually not recommended for biological images.
- 8. Cloning or copying objects into a digital image, from other parts of the same image or from a different image, is very questionable.
- 9. Intensity measurements should be performed on uniformly processed image data, and the data should be calibrated to a known standard.
- 10. Avoid the use of lossy compression.
- 11. Magnification and resolution are important.
- 12. Be careful when changing the size (in pixels) of a digital image.

These guidelines can also be found as part of the "Online Learning Tool for Research Integrity and Image Processing" <a href="http://www.uab.edu/researchintegrityandimages/">http://www.uab.edu/researchintegrityandimages/</a> or: <a href="http://ori.dhhs.gov/education/products/RIandImages/">http://ori.dhhs.gov/education/products/RIandImages/</a>

### Where to publish?



- Dryad
- Figshare
- GigaDB
- Github
- Zenodo
- MorphoBank
- MorphoDBase
- MorphoMuseuM
- MorphoSource

Type of data (volume, surface, analyses...)?
Size restriction?
Costs?

Type of license for re-use?
Globally unique identifier (DOI) offered?

#### What metadata should you publish?

S. Faulwetter, N. Minadakis, K. Keklikoglou, M. Doerr, C. Arvanitidis

First steps towards the development of an integrated metadata management system for biodiversity-related micro-CT datasets.

https://www.bruker.com/fileadmin/user\_upload/8-PDF-Docs/PreclinicalImaging/microCT/2015/uCT2015-27.pdf

Davies TG et al., Donoghue PCJ. (2017).

Open data and digital morphology.

Proceedings of the Royal Society B: Biological Sciences 284(1852).

http://rspb.royalsocietypublishing.org/content/royprsb/284/1852/20170194.full.pdf

### Science begins here:



and WONDER!